Amendments to the Specification:

Please replace the paragraph starting at page 13, line 23 with the following paragraph:

Purification of the lipase was performed from E.coli cells expressing the lipase in an appropriate vector. The purification essentially involves passing the cell lysate in phenyl-sepharose column followed by a Mono-S column. Lipase is an aggregated prone protein, care especially keeping protein concentration below 5mg/ml, was taken to avoid aggregation of the protein. The purification of the lipase was carried out essentially as described earlier[32] with minor modifications. Lipase from culture filterates of Bacillus strain or from the E.coli lysates was processed similarly. For purification of the wild type and mutant proteins from E.coli the lipA gene or the mutant genes are cloned into pET 21b.). For this, the gene corresponding to the full length, mature protein was amplified with primers PrNde I (forward primer) (5'-CCATGATTACGCATATGGCTGAACACAA-3')(SEQ ID No. 15) and JOF. The forward primer had an engineered Nde I site. The forward primer also introduced a start codon at the start of the lipase gene in the form of the ATG sequence that is part of the Nde I recognition sequence. This would introduce a methionine in the N-terminus of the mature protein, expressed in *E.coli*, just before the N-terminal alanine that occurs in the protein purified from the culture supernatant of *B.subtilis*. The wild type protein as well as the mutants were amplified, digested with Nde I and BamH I and ligated with pET-21b digested with Nde I and BamH I. The ligation mix was transformed into *E.coli* DH5α and the positives were selected by plasmid minipreps and restriction digestions (Fig.1 and Fig.2).

Please replace the paragraph starting at page 21, line 28 with the following paragraph:

The clone pLipA containing the complete lipA gene in pBR322 plasmid was a kind gift from Dr Frens Pierce (Fig.1). The lipase gene along with the region coding for the signal sequence was amplified with primers For1 (forward primer) (5'-GGAGGATCATATGAAATTTGTAAAAA-

3')(SEQ ID No. 16) and Rev1 (reverse primer) (5'-CCCGGGATCCATTGTCCGTTACC-3')(SEQ ID No. 17). The primers contained engineered Nde1 and Bam H1 sites respectively. The ATG of the Nde1 site in For1 coincided with the natural start codon of the lipase and the BamH1 site was beyond the natural stop codon. The amplified product was digested with Nde 1 and Bam H1 and cloned into the Nde 1-BamH1 sites of the plasmid pET-21b yielding the plasmid pET-lipwt (Fig.2) . The lipase gene coding for the mature protein was amplified from pET-lipwt by using primers PREcoR I (forward primer) (5'- CGTCAGCGAATTCCGCTGAACACAT-3')(SEQ ID No. 18) and PRBamH I (reverse primer) (5'-GCGGGAAGGATCCGAATTCGAGCT-3')(SEQ ID No. 19). The primers had an engineered EcoR I and BamH I site respectively. The amplified product was cleaved by EcoR I and BamH I and cloned into the EcoR1-BamH1 sites of the plasmid pJO290. This construct (pJ0290lip) was used for screening thermostable mutants (Fig.3). The *E.coli* strain JM109 was used for all the screening steps and all media contained 0.2 % glucose unless otherwise mentioned. This system was chosen because it allows low-level, controlled and inducible expression of the gene product in *E.coli,* which is necessary to prevent the reported toxicity of the protein to *E.coli* and to prevent complications from *in vivo* insolubility of this highly hydrophobic and aggregation-prone protein.

Please replace the paragraph starting at page 23, line 1 with the following paragraph:

The lipase gene was mutagenised by error-prone PCR (Cadwell and Joyce, 1992). Primers JOF (5'-CGCCAGGGTTTTCCCAGTCACGAC-3')(SEQ ID No. 20) and JOR (5'-TGACACAGGAAACAGCTATGAC-3')(SEQ ID No. 21) flank the gene beyond the Eco R1 and Bam H1 sites present on the plasmid. Error-prone PCR was carried out in a 100 µl reaction volume containing 20 femtomoles of the plasmid pJO290-lip, 50 pmoles each of primers JOF and JOR, 100 mM Tris.Cl (pH 8.3 at 25 °C), 500 mM KCl, 0.1 % gelatin (w/v), 7 mM $MgCl_2$, 0.25 mM $MnCl_2$, 1 mM each of dTTP and dCTP, 0.2 mM each of dATP and dCTP and 5 units Taq DNA polymerase. After an initial denaturation of 3 min at 94 °C, the following steps were repeated for 30 cycles in a thermal cycler: 1 min at 94 °C, 1 min at 45 °C and 1 min at 72 °C. The

amplified product was precipitated with ethanol, eluted from a 1 % agarose gel and digested with EcoR I and BamH I. The digested product was again eluted from a 1 % agarose gel and ligated with pJO290 digested with EcoR I and BamH I. The ligation mix was transformed into *E.coli* JM109 and selection was done on LB-agar supplemented with 25 μg/ml chloramphenicol and 0.2 % glucose.

Please replace the paragraph starting at page 23, line 1 with the following paragraph:

The mutant Gene sequence 6 (triple mutant) was created by site-directed mutagenesis on the Gene sequence 5 template using the mutagenic primer PROLF: 5'- GGC AAG GCG CCT CCG GGA ACA GAT- 3' (SEQ ID No. 22) to incorporate a codon change CTT → CCT that led to L114P change in the amino acid sequence. The sequences of all the genes were confirmed by automated DNA sequencing.